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Title: Biocrusts modulate responses of nitrous oxide and methane soil fluxes to simulated climate change in a Mediterranean dryland

Running title: Biocrusts, climate change and greenhouse gas fluxes

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23

24 **Abstract**

25 Little is known about the role of biocrusts in regulating the responses of N₂O and CH₄ fluxes to climate change in drylands. Here, we
26 aim to help filling this knowledge gap by using an eight-year field experiment in central Spain where temperature and rainfall are
27 being manipulated (~1.9 °C warming, 33% rainfall reduction, and their combination) in areas with and without well-developed
28 biocrust communities. Areas with initial high cover of well-developed biocrusts showed lower N₂O emissions, enhanced CH₄ uptake
29 and higher abundances of functional genes linked to N₂O and CH₄ fluxes compared with areas with poorly-developed biocrusts.
30 Moreover, biocrusts modulated the responses of gases emissions and related functional genes to warming and rainfall reductions.
31 Specifically, we found under rainfall exclusion and its combination with warming a sharp reduction in N₂O fluxes (~96% and ~197%,
32 respectively) only under well-developed biocrust cover. Warming and its combination with rainfall exclusion reduced CH₄
33 consumption in areas with initial low cover of well-developed biocrust, whereas rainfall exclusion enhanced CH₄ uptake only in areas
34 with high initial cover of well-developed biocrusts. Similarly, the combination of warming and rainfall exclusion increased the

35 abundance of the *nosZ* gene compared to the rainfall exclusion treatment and increased the abundance of the *pmoA* gene compared to
36 the control, but only in areas with low biocrust cover. Taken together, our results indicate that well-developed biocrust communities
37 could counteract the impact of warming and altered rainfall patterns on soil N₂O and CH₄ fluxes, highlighting their importance and the
38 need to preserve them to minimize climate change impacts on drylands.

39 **Keywords**

40 Biocrust, *denitrifiers*, dryland, methane, *methanotrophs*, nitrous oxide.

41

42 **Highlights**

- 43 • Under the combination of rainfall exclusion and warming, biocrusts reduced N₂O fluxes.
- 44 • Biocrusts enhanced the rate of CH₄ uptake.
- 45 • Soils with high biocrust cover had higher abundances of *pmoA* and *nosZ* genes.

46 **Introduction**

47 Most efforts to understand the main drivers of soil greenhouse gas (GHG) fluxes under global change scenarios have focused on
48 carbon dioxide (CO₂) (Pachauri and Meyer 2014). Much less is known about other greenhouse gases such as nitrous oxide (N₂O), and
49 methane (CH₄), which have stronger greenhouse effects and can significantly affect feedback responses to climate change
50 (Nakicenovic and Swart 2000; Le Mer and Roger 2001; Soussana and others 2007; Oertel and others 2016). This is especially true for
51 dryland (arid, semi-arid and dry-subhumid) ecosystems, which cover ~45% of the land surface (Právělie 2016) and sustain over 40%
52 of human population (Reynolds and others 2007). The exchange of N₂O and CH₄ between the soil and the atmosphere has been
53 traditionally considered of little importance in drylands due to their typically low water and nutrient contents, which limit biological
54 activity (Dalal and Allen 2008). However, over the last two decades multiple studies have reported elevated N₂O fluxes in dryland
55 soils after rainfall pulses (Barton and others 2008, 2013; Zaady and others 2013), and have noted their potential as a relevant global
56 sink of atmospheric CH₄ (Potter and others 1996; Angel and Conrad 2009). Furthermore, the relevance of drylands as a contributor to
57 the global balance of GHG fluxes will increase in the future, as their global extent will likely increase by 11-23% by the end of this
58 century due to climate change (Huang and others 2016). However, how warming and forecasted changes in rainfall patterns will affect
59 N₂O and CH₄ fluxes in drylands remains poorly studied (Darrouzet-Nardi and others 2015; Guan and others 2019).

60 Soil N₂O and CH₄ transformations are largely carried out by highly specialized microbial communities. For instance, the N₂O
61 produced in both nitrification and denitrification processes (Firestone and Davidson 1989; Bremner 1997; Canfield and others 2010) is
62 reduced to N₂ by the *nosZ* carrying denitrifiers under anaerobic conditions (Bremner 1997; Canfield and others 2010). In dryland

63 surface soils, aerobic nitrification has been traditionally considered the dominant process (Delgado-Baquerizo and others 2016).
64 Consequently, the *nosZ* gene (carried by denitrifying bacteria) and the factors affecting its abundance and activity have been poorly
65 studied (Philippot and others 2007; Hallin and others 2018). However, aggregates and precipitation pulses create anaerobic conditions
66 favourable for denitrification in dryland soils, which could represent a temporary sink for atmospheric N₂O, the substrate used by *nosZ*
67 denitrifiers (Austin and others 2004; Ley and others 2018; Wang and others 2019). Likewise, under aerobic conditions (dominant in
68 dryland soils), CH₄ oxidizing bacteria use the CH₄ monooxygenase (encoded by the *pmoA* gene) to oxidize CH₄, constituting the only
69 biological sink for atmospheric CH₄ (Dalal and Allen 2008; Conrad 2009). Previous experiments and observational studies (Nazaries
70 and others 2013; Powell and others 2015) have shown strong relationships between the abundance of *nosZ/pmoA* genes and GHG
71 fluxes, and consequently functional genes have been used to predict these fluxes (Nazaries and others 2013; Powell and others 2015).
72 Unfortunately, most of our knowledge on *nosZ* and *pmoA* genes comes from mesic ecosystems (Nazaries and others 2013; Powell and
73 others 2015; Martins and others 2017; but see Martins and others 2015, Lafuente and others 2019), and we lack studies evaluating the
74 changes in their abundance under global change scenarios in drylands.

75 Biocrusts, soil surface communities composed by lichens, mosses, liverworts, fungi, algae, cyanobacteria and other
76 microorganisms, are a key biological component of dryland ecosystems worldwide (Weber and others 2016). Biocrusts regulate a
77 myriad of key soil biotic and abiotic properties and processes (Eldridge and others 2010; Aschenbach and others 2013; Maestre and
78 others 2013; Zaady and others 2013; Felde and others 2014), and are home to particular soil microbial communities (Steven and others
79 2013; Delgado-Baquerizo and others 2018). However, and to the best of our knowledge, no previous field studies have experimentally

evaluated how biocrusts influence soil N₂O and CH₄ fluxes under simulated climate change. Such studies are needed not only to advance our understanding of climate change impacts on drylands, where biocrusts are a prevalent biotic feature, but also to provide relevant data to refine simulation models employed to forecast future N₂O and CH₄ fluxes across dryland biomes.

Herein, we used an eight-year (2008-2016) warming and rainfall manipulation experiment located in central Spain (Maestre and others 2013) to investigate: (i) the effects of simulated climate change (~1.9 °C warming and ~33% rainfall reduction) on soil N₂O and CH₄ fluxes and the abundance of *nosZ* and *pmoA* functional genes; (ii) whether these effects are modulated by biocrusts; and (iii) the relationships between N₂O and CH₄ fluxes and the abundance of *nosZ* and *pmoA* functional genes, respectively.

Materials and methods

Study site

This experiment was conducted in the Aranjuez Experimental Station (central Spain; 40°01'55.7''N-3°32'48.3''W; 590 m.a.s.l; for more details on this experimental station see <http://maestrelab.blogspot.com/2013/05/the-aranjuez-experimental-station.html>). Its climate is Mediterranean semi-arid, with average annual temperature and rainfall of 15°C and 358 mm, respectively (data available since 1977 from the Aranjuez Meteorological Station, 40°04'N - 3°32'W; 540 m.a.sl). Soils are gypsum-derived (Gypsic Leptosols, WRB 2006). Organic carbon (C), total nitrogen (N), and pH vary among the considered microsites (i.e. areas with low and high biocrust cover) between 1.8-5.0%, 0.14-0.44%, and 6.6-7.2, respectively. Vegetation is dominated by *Macrochloa tenacissima* (L.) Kunth (18% of total cover), *Retama sphaerocarpa* (L) Boiss and *Helianthemum squamatum* Pers. (6% of total cover for both shrubs).

97 Open areas between vascular plants are partially covered with a well-developed biocrust community dominated by lichens such as
98 *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia*
99 (Scop.) Timdal, and *Psora decipiens* (Hedw.) Hoffm, which covers ~34 % of the soil surface (see Maestre and others 2013 for a full
100 species checklist).

101 *Experimental design*

102 A detailed description of the experimental design can be found in Escolar and others 2012. Briefly, we established a fully factorial
103 experimental design with three factors, each with two levels: warming (control vs. ~1.9 °C soil temperature increase), rainfall
104 exclusion (control vs. 33% rainfall reduction) and biocrust cover (<25% vs. >50% of lichens and mosses; hereafter low and high
105 biocrust cover, respectively). To simulate warming, we used 40 × 50 × 32 cm hexagonal open top chambers (OTCs) made of
106 methacrylate, which were elevated 5 cm from the surface to avoid overheating (Fig. S1a). To intercept rainfall, we built a 1.2 × 1.2 × 1
107 m metallic frame supporting three V-shaped methacrylate (Fig. S1b) that covered ~37% of the canopy at warming and rainfall
108 exclusion treatments were setup in July and November 2008, respectively (see Maestre et al. 2013 for additional details). Ten
109 replicates per combination of treatment were established, resulting in 80 experimental plots. Our warming treatments (warming and its
110 combination with rainfall exclusion), significantly increased soil temperatures by ~1.6°C and 2.3°C, respectively, compared to control
111 plots (average of the 2008-2019 period). Our rainfall exclusion (RE) shelters excluded on average ~33% of the incoming rainfall
112 (2008-2013 period).

113 At each plot we inserted a polyvinyl chloride (PVC) ring (diameter = 20 cm, height = 7 cm) 5 cm into the ground at the start of
114 the experiment for measuring GHG fluxes and monitoring changes in biocrust cover. Well-developed biocrust cover (i.e. lichens and
115 mosses) was estimated annually using high resolution pictures since the setup of the experiment in 2008, as detailed in Maestre and
116 others (2013). Values obtained using these pictures are highly correlated with those obtained with *in situ* surveys (Ladrón de Guevara
117 and others 2018).

118 Soil moisture (0-5 cm) and temperature (0-2 cm) were monitored every 2.5 h and 0.5 h, respectively in all treatments in a
119 subset of the plots using EC-5 soil moisture (Decagon Devices Inc., Pullman, WA, USA) and HOBO® TMC20 (Onset Corporation,
120 Bourne, MA, USA; Figs. S2 and S3) sensors.

121 *Greenhouse gas exchange measurements*

122 We estimated soil-atmosphere N₂O and CH₄ fluxes in seven replicates per combination of treatments using the static chamber method
123 (Bowden and others 1990). From March 2015 to May 2016, 14 sampling campaigns were carried out approximately once a month.
124 Immediately before each measurement, a 20 cm diameter and 9 cm high PVC chamber was placed on top of each of the 56 permanent
125 rings and sealed with a rubber band. Each chamber had a sampling port in the top centre that allowed air sampling and was covered
126 with reflective material to thermally isolate it during the measurement. Gas samples were collected at 0, 30 and 60 min after chamber
127 closure using a needle attached to a polypropylene syringe, transferred to 22 ml pre-vacuumed vials and kept at room temperature until
128 analysis. We estimated N₂O and CH₄ concentrations in the gas samples using a HP-6890 gas chromatograph (GC), equipped with a

129 headspace autoanalyzer (HT3) (Agilent Technologies, Barcelona, Spain), a ^{63}Ni electron capture detector (for N_2O), and a flame-
130 ionization detector fitted with a methaniser (for CH_4 detection). The carrier gas used was helium.

131 *Soil sampling and analyses*

132 Soil samples (0-2 cm) were collected five times during the study period (June, September and November 2015 and February and April
133 2016) from five replicates per combination of treatments. Each soil sampling always matched one of the gas measurement campaigns.
134 Visible biocrusts (i.e. lichens and mosses) were removed when present and then soils were stored in $-20\text{ }^\circ\text{C}$ for DNA extractions.

135 Total genomic DNA was extracted from 0.6 g of frozen soil using the PowerSoil DNA Isolation kit (MOBIO Laboratories, Inc.
136 USA) according to manufacturer's protocol but with a slight modification during the cell lysis step (we used a tissue homogenizer
137 [Precellys 24- dual. Bertin technologies, France] at a speed of 4500 rpm for 45 s, twice). DNA extractions yields ranged from 0.1 to
138 132.6 ng/ μl , with an average of 6 ± 15 ng/ μl (mean \pm standard deviation, $n = 5$). The abundances of *nosZ* and *pmoA* genes were
139 determined using *nosZ2f/nosZ2r* (Henry and others 2006) and *pmo189f/pmo650r* (Bourne and others 2001) primers, respectively. All
140 primers were purchased from Integrated DNA Technologies (Australia). Each sample was quantified (in duplicate) in a total volume of
141 10 μl using a BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). The reaction mixture
142 contained 1 μl of DNA template (2 ng/ μl ; those samples with a concentration <2 ng/ μl were not diluted in sterilised water), 5 μl of
143 SensiFast Sybr No-Rox Mix (2x) (Bioline, Australia), 0.3 μl of each primer (0.4 mM) and 0.4 μl of BSA (0.4 mg/ml). Thermal cycling
144 conditions and primer sequences can be found in Table S1. The *nosZ* gene was cloned with pGEM-T Easy Vector kit according to
145 manufacturer's instructions (Promega, Madison, USA) and transformed into *Escherichia coli* strain JM109 to perform calibration

curves. The *pmoA* gene calibration curves were made from genomic DNA (*Methylosinus trichosporium*). Melt curve analyses were performed in each assay to verify the specificity of the amplicon products. Gene copy number per g dry soil normalized to extraction yield were calculated for both genes.

Statistical analyses

We estimated N₂O and CH₄ fluxes as described in Durán and others (2013), and reported them as changes in milligrams or micrograms (for N₂O) per square meter per day. In more than 90% of the cases, the increases in N₂O and CH₄ emissions were linear ($R^2 > 0.7$). Non-linear rates were discarded, and imputation of missing rates (per treatment) was performed using the missForest algorithm in the R package missForest (Stekhoven and Bühlmann 2012), which iteratively fills missing values in all columns of a data frame based on predictions from random forest models. For the iteration, we included the averaged soil moisture and temperature matching the treatment, date and time of the sampling. We estimated the 2.3% and 6.8% of the N₂O and CH₄ rates analysed in this study, respectively.

We first tested the effects of warming, rainfall exclusion and biocrust cover (i.e. low and high biocrust cover in the ring when the experiment was established in 2008) on N₂O and CH₄ fluxes and soil microbial gene abundance (*nosZ* and *pmoA* functional genes) with a repeated measures general linear mixed effects model. We also included the rate of change in the biocrust cover over time (in %) as a covariate in the models to control for the observed changes in biocrust cover since the setup of the plots in 2008 (described in detail in Ladrón de Guevara and others 2018). As multiple interactions between initial biocrust cover and the climate change treatments were found (Table 1), we tested the effect of warming and rainfall exclusion (alone and combined) separately for low and

high biocrust cover plots using the same model. These analyses were carried out using the function *lmer* in the R package *lmer4* (Bates and others 2015). Differences of least squares means for the factors of the mixed effects model were calculated using the function *diffLsmeans* in the R package *lmerTest* (Kuznetsova 2017) with no p-value adjustment. We compared differences in gas fluxes and gene abundances between the two levels of biocrust cover (low and high), with the student's t-test. Methane fluxes and soil microbial gene abundances were log transformed prior to analyses to improve normality. All statistical analyses were performed using R statistical software 3.4.0 (R Core Team 2017). Data are available on Figshare (Lafuente and others 2020).

Results

Effects of simulated climate change on N₂O and CH₄ fluxes

Nitrous oxide fluxes were very low in all cases, ranging on average from -10 to 20 $\mu\text{g m}^{-2} \text{d}^{-1}$, and had a high temporal variability (Figs. S4a,b, and 1a,b). These fluxes did not differ between biocrust cover levels (Fig. 1 a,b; $p=0.14$; Fig. S5a). Biocrusts, however, regulated the responses of N₂O emissions to warming and rainfall exclusion. In low biocrust cover plots, these climatic manipulations reduced N₂O fluxes (vs. control) in March, April and early July, and increased them in late July and September (Fig. S4a). However, in high cover plots we observed sharp reductions in N₂O fluxes in the rainfall exclusion and warming + rainfall exclusion treatments as compared with the control plots (~96% and ~197%, respectively; Fig. 1b, $p < 0.05$, Table S2).

Methane fluxes were also low and negative (i.e. CH₄ uptake) in most cases, and ranged on average from -1.66 to -1.22 $\text{mg m}^{-2} \text{d}^{-1}$ (Figs. S4c,d and 1c,d). The CH₄ uptake was higher in high (vs. low) biocrust cover plots ($p<0.01$; Fig. S5b). The response of CH₄

180 fluxes to warming and rainfall exclusion was very variable throughout the study period, and was modulated by biocrust cover (Fig.
181 1c,d). All climate change treatments tended to decrease CH₄ uptake in low biocrust cover plots, although these differences were only
182 found in the warming and warming + rainfall exclusion treatments (Table S2, Fig. 1c). However, in high biocrust cover plots, only
183 warming reduced CH₄ uptake (Table S2, Fig. 1d).

184 *Effects of climate manipulation on the abundances of nosZ and pmoA genes*

185 Both *nosZ* and *pmoA* genes were more abundant in high than in low biocrust cover plots ($p < 0.05$; Fig. S5c,d). As found with N₂O and
186 CH₄ fluxes, we observed a marked variability in *nosZ* and *pmoA* gene abundance throughout the experiment (Fig. S6), as well as
187 important differences in their responses to warming and rainfall exclusion treatments depending on biocrust cover (Figs. S6 and 2).

188 The averaged abundances of the *nosZ* gene ranged from 2.4×10^5 to 7.8×10^7 copy number g dry soil⁻¹ (Table S3, Fig. 2a, b).
189 Warming, rainfall exclusion, and their combination reduced the abundance of the *nosZ* gene (vs. the control) in the September
190 sampling. In low biocrust cover plots, its overall abundance was higher at the warming + rainfall exclusion treatment than at the
191 rainfall exclusion treatment (Table S3, Fig. 2a). We did not find any relationship between N₂O fluxes and *nosZ* gene abundance ($R^2 =$
192 0.00; $p = 0.91$ and $R^2 = 0.02$; $p = 0.07$ in low and high biocrust cover plots, respectively).

193 On average, the *pmoA* gene abundance ranged from 1.6×10^4 to 8.3×10^5 copy number g dry soil⁻¹ (Fig. 2c,d). The combination
194 of warming and rainfall exclusion led to an overall increase in the abundance of the *pmoA* gene, but only in the low biocrust cover
195 plots (Fig. 2c,d; Table S3). A positive relation between CH₄ fluxes and the abundance of the *pmoA* gene was observed in the warming
196 + rainfall exclusion plots, but only in low biocrust cover plots ($R^2 = 0.13$; $p = 0.04$).

197

198 **Discussion**

199 Our study provides novel experimental evidence that biocrusts are key regulators of the responses of N₂O and CH₄ fluxes and
200 associated functional genes to climate change drivers. Biocrusts regulated the temporal patterns of N₂O and CH₄ fluxes and their
201 response to our climate change treatments. For instance, and despite being highly variable in space and time, the combination of
202 warming and rainfall exclusion led to a sharp reduction (197%) in average N₂O fluxes, but only in areas with high biocrust cover.
203 Biocrusts also mitigated reductions in CH₄ uptake observed under the combination of warming and rainfall exclusion. These results
204 highlight the importance of considering biocrusts when assessing ecosystem responses to climate change in drylands and when
205 estimating future GHG fluxes from soils in these ecosystems, which are forecasted to cover more than 50% of the terrestrial surface by
206 the end of this century (Huang and others 2016).

207 Our results suggest that projected changes in temperature and precipitation will likely modify the capacity of dryland soils to
208 exchange N₂O with the atmosphere. More importantly, these findings indicate that such responses depend on the degree of biocrust
209 development. While in low biocrust cover areas rainfall exclusion (and its combination with warming) tended to increase N₂O
210 emissions throughout the study period, this treatment promoted a sharp decrease in these emissions when well-developed biocrust
211 communities were present. Furthermore, soils at high biocrust cover plots were a net N₂O sink under the combination of warming and
212 rainfall exclusion. These results highlight the ability of biocrusts to mitigate the effects of climate change on N₂O emissions, but also
213 the importance of considering the interactions among different climate change drivers when evaluating potential future GHG emissions

214 (Fig. S4b). Interestingly, and despite the reductions in biocrust cover induced by warming over the years in our experiment (Ladrón de
215 Guevara and others 2018), we still found a sharp reduction in N₂O fluxes in the rainfall exclusion and its combination with warming
216 treatments. Such a result suggests a strong legacy effect of biocrusts on soil functioning, similar to that reported in other mesic
217 ecosystems with plants (Meisner and others 2013), and further highlights the importance of these communities on driving the
218 responses of drylands to climate change drivers.

219 Climate change effects on N₂O fluxes are highly variable due to the key importance of climatic factors such as soil temperature
220 and moisture as drivers of GHG emissions (Dijkstra and others 2012; Zhou and others 2016). Warming, and the associated increases in
221 soil temperature, could enhance the metabolism of nitrifiers and denitrifiers, boosting N₂O emissions (Dalal and others 2003) (Fig.
222 S3). However, and particularly in drylands, climate change-driven reductions in soil moisture (either associated with warming or due
223 to decreases in precipitation) can limit microbial metabolism and thus reduce atmospheric N₂O emissions (Chapuis-Lardy and others
224 2007). Overall, our rainfall exclusion and warming treatments promoted soil drying, as shown in Escolar and others (2012) and
225 Maestre and others (2013). A detailed analysis of soil moisture changes after rainfall events in our experiment showed how biocrusts
226 increased water gains after rainfall events but enhanced soil desiccation after rainfall pulses (Lafuente and others 2018). Thus, the
227 reductions in water availability due to our climate change treatments, particularly in areas with high biocrust cover, might explain the
228 decreases in N₂O emissions observed in these plots (Fig. S2). Alternatively, nutrient availability is also often highlighted as a key
229 driver of dryland N₂O fluxes (Dalal and Allen 2008; Dijkstra and others 2013). Under aerobic conditions and high availability of N
230 substrate (i.e. NH₄⁺), nitrification is expected to dominate over denitrification (Weier and others 1993; Dalal and others 2003), which

231 have been reported to lead to an accumulation of inorganic N forms in global drylands (Delgado-Baquerizo and others 2016). More
232 importantly, we have found in our experiment that warming + rainfall exclusion treatments often lead to an accumulation of inorganic
233 N in low (not in high) biocrust cover plots (Delgado-Baquerizo and others 2014). Similarly, previous studies at our experimental site
234 have found a higher potential nitrification rate and available NO_3^- in bare soil areas compared to areas with well-developed biocrusts
235 (Castillo-Monroy and others 2010), where DON is the dominant N form (Delgado-Baquerizo and others 2010). Thus, in drylands,
236 having a well-developed biocrust community could be linked to a lower accumulation of inorganic N (Delgado-Baquerizo and others
237 2014), therefore limiting the availability of substrate for the denitrification process and ultimately reducing N_2O fluxes to the
238 atmosphere from incomplete denitrification leaks (Dalal and Allen 2008).

239 It is important to highlight the importance of the selected denitrification gene studied. Under aerobic conditions, nitrification
240 produces N_2O as a by-product (Bremner 1997; Canfield and others 2010), a process that is expected to be important in drylands given
241 their reported relatively high mineralization rates. However, denitrification is an anaerobic multistep process that also produces N_2O
242 (Firestone and Davidson 1989). Anaerobic soils are not dominant in drylands, but favourable conditions for denitrification can be
243 created in soil aggregates or after precipitation pulses (Austin and others 2004; Ley and others 2018). The last step of the
244 denitrification pathway consists on the conversion of N_2O into N_2 , a step catalysed by the nitrous oxide reductase codified by the
245 functional gene *nosZ* (Philippot and others 2007). Consequently, the *nosZ* gene has been used to estimate N_2O fluxes (Powell and
246 others 2015). Our climate change treatments had no detectable effects on this gene regardless the initial biocrust cover considered.
247 However, the abundance of the *nosZ* gene tended to increase in the warming and rainfall exclusion treatments only in high biocrust

cover plots (Fig. 2a, b). This may also help explain, at least partially, the average lower rates of N₂O observed in these plots throughout the whole study. However, we cannot obviate that (i) we have evaluated functional genes at DNA level, and consequently we cannot know whether this gene is being expressed or not; and (ii) the primers used, which fail to amplify *nosZ* clade II gene (Jones and others 2013), have recently been described to be abundant in soils and thus an important contributor to N₂O fluxes (Domeignoz-Horta and others 2016; Stein 2017; Hallin and others 2018).

Our climate change treatments consistently and relatively reduced CH₄ uptake, as found in another study carried out in a semiarid grassland (Dijkstra and others 2013). Methane oxidation requires gas diffusivity to provide atmospheric CH₄ to soil methanotrophs, a step catalysed by the CH₄ monooxygenase codified by the *pmoA* gene (Dalal and Allen 2008). In more mesic ecosystems, decreased soil moisture would improve gas diffusivity, increase soil aeration and CH₄ oxidation. However, drylands are water limited ecosystems, so further reductions in soil moisture by our climate change treatments might have limited the activity of CH₄ oxidizing bacteria (Schnell and King 1996; Galbally and others 2008; Sullivan and others 2013) (Figs. S2 and S3). Similarly, increased temperatures have been described previously to drive changes in the community composition of CH₄ oxidizing bacteria (Mohanty and others 2007), which can also help to explain the differences in CH₄ uptake observed among treatments (Nazaries and others 2013). Interestingly, we observed a positive correlation between changes in biocrust cover during the lifetime of our experiment and CH₄ uptake. Put simply, the loss of cover through time observed in high biocrust cover plots (Ladrón de Guevara and others 2018) was linked to decreases in CH₄ uptake (Fig. S7). Methane oxidation is very sensitive to changes in temperature and more importantly in moisture, changes related to water stress and gas diffusivity (Smith and others 2000). Thus, it is likely that the known changes

265 exerted by biocrusts in soil properties and processes (Barger and others 2016; Chamizo and others 2016; Weber and others 2016),
266 might have improved the environmental conditions (e.g. mitigating water and heat stress) for methanotrophs or changed the CH₄
267 oxidising bacterial community, affecting CH₄ uptake, even in deeper layers where most CH₄ uptake occurs (Butterbach-Bahl and
268 Papen 2002).

269 The abundance of the *pmoA* gene was higher in high than in low biocrust cover plots. The well-known positive impacts of
270 biocrusts on soil fertility (Weber and others 2016) could underlie this increase in microbial abundance (Maestre and others 2011;
271 Barger and others 2016), which in turn might have contributed, at least partially, to increase the overall CH₄ uptake observed during
272 the entire duration of this study (Le Mer and Roger 2001). However, and in contrast with a previous study carried out in an Australian
273 forest that found correlated gene abundances and GHG emissions (Martins and others 2016), we could not find a relationship between
274 the overall abundance of the *pmoA* gene and overall CH₄ uptake. Methane uptake depends on the balance between gas diffusivity and
275 metabolic stress (Luo and others 2013). Thus, our results can be the consequence of microbial activity limitation due to water stress.
276 Indeed, in low biocrust cover plots, we detected an increase in *pmoA* abundance in the warming + rainfall exclusion treatment (Fig.
277 2c). Despite such increase, this treatment did not show enhanced CH₄ uptake, which supports that reductions in soil moisture could
278 have limited microbial metabolism. Alternatively, we cannot discard that the interference of soil NH₄⁺, which competes with CH₄ for
279 the methane monooxygenase (King and Schnell 1994), could be behind the observed lack of correlation between *pmoA* abundance and
280 CH₄ uptake.

Together, our findings highlight how biocrusts are essential regulators of soil-atmosphere N₂O and CH₄ fluxes and their responses to simulated climate change, directly and indirectly by improving soil environmental conditions (i.e. reducing water and heat stress) for N₂O reducers and methanotrophs. They also show that functional microbial abundance (i.e. *nosZ* and *pmoA* carrying bacteria) can also be highly variable in time, providing evidence for seasonal patterns in these functionally important bacterial communities. Our results also illustrate how biocrusts affect temporal patterns in the fluxes of N₂O and CH₄ and associated functional genes. On average, the “biocrust legacy” reduced the rate of N₂O emissions, increased the rate of CH₄ uptake and increased the abundance of both *nosZ* and *pmoA* genes. More importantly, biocrusts mitigated the reductions in CH₄ uptakes observed under the combination of warming and rainfall exclusion treatments. Our findings emphasize the importance of well-developed biocrust communities to mitigate the impacts of warming and altered rainfall patterns on the emission of GHG fluxes from dryland soils, and thus the need to preserve them to minimize the negative consequences of ongoing climate change and to maintain ecosystem functioning in a warmer and drier world.

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469

470 **Table 1.** Linear mix model of the effect of climate change treatments on N₂O and CH₄ fluxes (n = 7) and functional gene abundances
471 (n=5). The rate of change in the biocrust cover over time (Δ BSC, in %) has been included in the models as a covariate to control for
472 the observed changes in biocrust cover since the setup of the plots in 2008. WA = warming, RE = rainfall exclusion, BSC = biocrust
473 cover, Num = Numerator degrees of freedom and Den = denominator degrees of freedom.

	N ₂ O				CH ₄				<i>nosZ</i>				<i>pmoA</i>			
	Num	Den	F	P	Num	Den	F	P	Num	Den	F	P	Num	Den	F	P
Warming	1	51.19	0.55	0.461	1	52.96	6.88	0.011	1	31.60	0.61	0.440	1	32.13	3.30	0.079
Rainfall Exclusion	1	47.60	1.56	0.218	1	48.45	0.42	0.522	1	30.42	0.10	0.755	1	30.66	0.00	0.986
BSC	1	51.29	1.37	0.248	1	53.08	9.22	0.004	1	31.10	9.56	0.004	1	31.68	12.87	0.001
WA:RE	1	47.60	0.53	0.471	1	48.45	1.28	0.263	1	30.37	1.81	0.188	1	30.67	0.03	0.854
WA:BSC	1	50.13	0.02	0.896	1	51.61	0.66	0.419	1	30.66	0.10	0.753	1	31.08	0.94	0.339
RE:BSC	1	47.67	12.02	0.001	1	48.55	7.78	0.007	1	30.10	0.00	0.952	1	30.39	0.07	0.793
WA:RE:BSC	1	47.64	1.89	0.176	1	48.51	1.48	0.230	1	30.08	6.20	0.018	1	30.40	1.23	0.276
Δ BSC	1	72.47	0.16	0.686	1	84.66	2.66	0.106	1	40.22	0.23	0.637	1	41.33	0.62	0.436

474

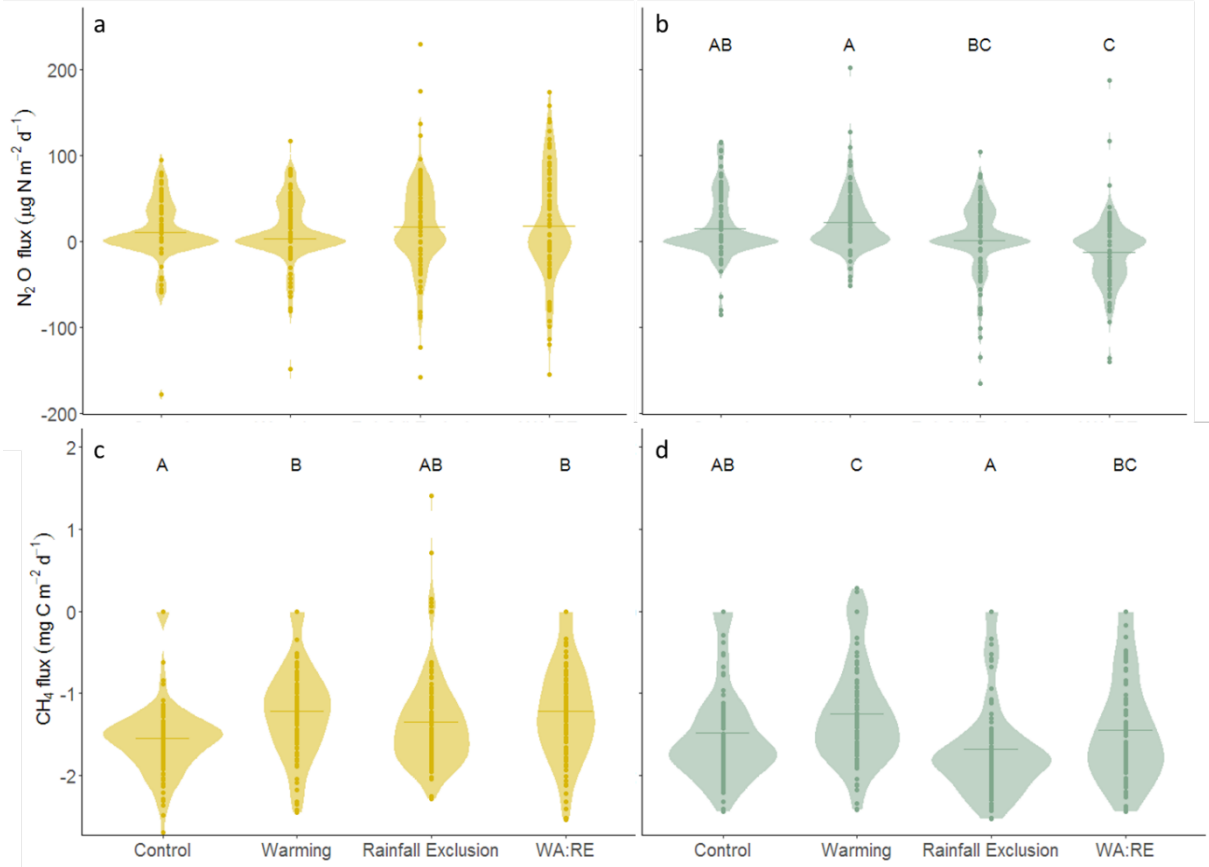
475

Figure legends

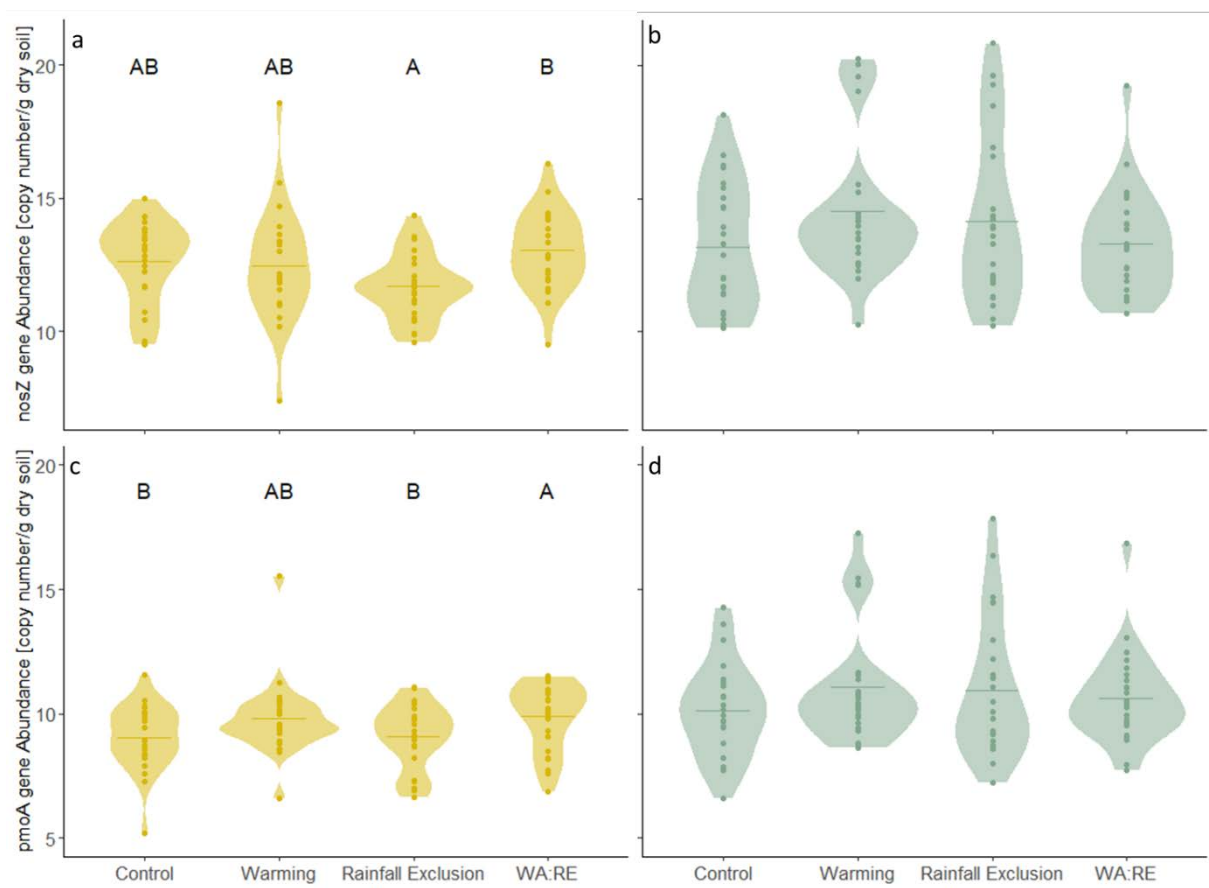
Figure 1. N₂O (a, b) and CH₄ (c,d) fluxes estimated in areas with low (left) and high (right) initial biocrust cover across the climate change treatments evaluated, the horizontal line shows the mean ($n=98$). The width of shaded area in the violin plot represents the kernel probability density (proportion of the data located there). Different letters indicate differences in pairwise comparisons among treatments by differences of least square means ($P<0.05$). WA:RE = warming and rainfall exclusion combined.

Figure 2. Log-transformed abundances of *nosZ* (a,b) and *pmoA* genes (c,d) in areas with low (left) and high (right) initial biocrust cover across the climate change treatments evaluated, the horizontal line shows the mean ($n=25$). The width of shaded area in the violin plot represents the kernel probability density (proportion of the data located there). Different letters indicate differences in pairwise comparisons among treatments by differences of least square means ($P<0.05$). WA:RE = warming and rainfall exclusion combined.

Figure 1



493 **Figure 2**



494